PB81-191140

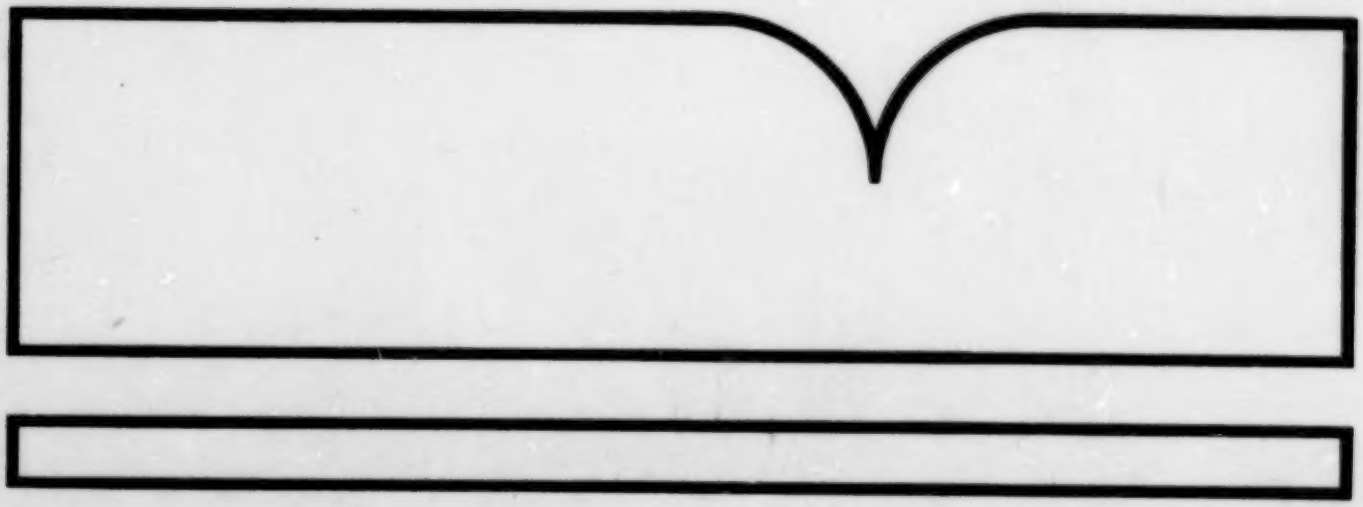
# Teratology and Postnatal Studies in Rats of the Propylene Glycol Butyl Ether and Isooctyl Esters of 2,4-Dichlorophenoxyacetic Acid

Midwest Research Inst.
Kansas City, MO

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Apr 81



U.S. Department of Commerce
National Technical Information Service
NTIS

PB81-191140

EPA 600/1-81-035
April 1981

# TERATOLOGY AND POSTNATAL STUDIES IN RATS OF THE PROPYLENE GLYCOL BUTYL ETHER AND ISOOCTYL ESTERS OF 2,4-DICHLOROPHENOXYACETIC ACID

by

Timothy M. Unger, Janet Kliethermes, Dan Van Goethem
and Robert D. Short
Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

DO NOT PHOTOGRAPH THIS PAGE

FINAL REPORT

Contract No. 68-02-2982
MRI Project No. 4604-B(3)

Project Officer

William F. Durham
Environmental Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

**TECHNICAL REPORT DATA**
*(Please read Instructions on the reverse before completing)*

| 1. REPORT NO. | 2. | 3. RECIPIENT'S ACCESSION NO. |
|---|---|---|
| EPA-600/1-81-035 | ORD Report | PB81-191140 |

**4. TITLE AND SUBTITLE**
Teratology and Postnatal Studies in Rats of the Propylene Glycol Butyl Ether and Isooctyl Esters of 2,4-Dichlorophenoxyacetic Acid

**5. REPORT DATE**
April 1981

**6. PERFORMING ORGANIZATION CODE**

**7. AUTHOR(S)**
Timothy M. Unger, Janet Kliethermes, Daniel Van Goethem, and Robert D. Short

**8. PERFORMING ORGANIZATION REPORT NO.**

**9. PERFORMING ORGANIZATION NAME AND ADDRESS**
Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

**10. PROGRAM ELEMENT NO.**
1EA615

**11. CONTRACT/GRANT NO.**
68-02-2982

**12. SPONSORING AGENCY NAME AND ADDRESS**
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Office of Research and Development
Research Triangle Park, North Carolina 27711

**13. TYPE OF REPORT AND PERIOD COVERED**

**14. SPONSORING AGENCY CODE**
EPA 600/11

**15. SUPPLEMENTARY NOTES**

**16. ABSTRACT**

The purpose of this study was to evaluate the teratogenic potential of the propylene glycol butyl ether (PGBE) and isooctyl (IO) esters of 2,4-dichlorophenoxyacetic acid (2,4-D). Accordingly, groups of pregnant CD® rats received daily oral doses of PGBE or IO equivalent to 0, 6.25, 12.5, 25.0, or 87.5 mg/kg/day of 2,4-D from day 6 through day 15 of gestation, and fetuses were observed for gross, soft tissue, and skeletal defects. In addition, a postnatal study was performed on rats receiving 0, 12.5, or 87.5 ME/kg/day PGBE or IO to determine the effect of treatment on growth and survival of pups. No adverse effects were observed on maternal welfare, nor was there any evidence of embryo or fetal lethality in any of the treated groups. Of the anomalies observed, the incidence of lumbar (14th) rib buds was found to be statistically increased in the groups given the 87.5 mg/kg/day doses of both PGBE and IO. No other anomaly reached a level of statistical or toxicological significance.

The number of pups per litter was significantly reduced on postpartum days 4 and 7 in dams receiving 87.5 ME/kg/day IO. However, mean pup body weight remained normal. Postnatal growth and survival of pups receiving PGBE were not adversely affected. It was concluded that PGBE and IO caused minor embryotoxicity which was not deleterious to growth and survival, and therefore was not teratogenic to offspring of treated rats.

**17. KEY WORDS AND DOCUMENT ANALYSIS**

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Toxicology<br>Teratology<br>Herbicide | Propylene Glycol Butyl Ether Ester<br>Isooctyl Ester<br>2,4-Dichlorophenoxyacetic Acid Esters<br>Rats | 06F,T |

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS *(This Report)* | 21. NO. OF PAGES |
|---|---|---|
| Release to Public | UNCLASSIFIED | 27 |
| | 20. SECURITY CLASS *(This page)* | 22. PRICE |
| | UNCLASSIFIED | |

## DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, US Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the US Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

## FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This study was undertaken to reevaluate the teratogenic potential of 2,4-D isooctyl (IO) and 2,4-D propylene glycol butyl ether (PGBE). These ester derivatives of the herbicide 2,4-dichlorophenoxyacetic acid(2,4-D) are commonly used for brush and weed control.

F.G. Hueter, Ph.D., Director
Health Effects Research Laboratory

## ABSTRACT

The purpose of this study was to evaluate the teratogenic potential of the propylene glycol butyl ether (PGBE) and isooctyl (IO) esters of 2,4-dichlorophenoxyacetic acid (2,4-D). Accordingly, groups of pregnant CD rats received daily oral doses of PGBE or IO equivalent to 0, 6.25, 12.5, 25.0, or 87.5 mg/kg/day of 2,4-D from day 6 through day 15 of gestation, and fetuses were observed for gross, soft tissue, and skeletal defects. In addition, a postnatal study was performed on rats receiving 0, 12.5, or 87.5 ME/kg/day PGBE or IO to determine the effect of treatment on growth and survival of pups. No adverse effects were observed on maternal welfare, nor was there any evidence of embryo or fetal lethality in any of the treatment groups. Of the anomalies observed, the incidence of lumbar (14th) rib buds was found to be statistically increased in the groups given the 87.5 mg/kg/day doses of both PGBE and IO. No other anomaly reached a level of statistical or toxicological significance.

The number of pups per litter was significantly reduced on postpartum days 4 and 7 in dams receiving 87.5 ME/kg/day IO. However, mean body weight remained normal. Postnatal growth and survival of pups receiving PGBE were not adversely affected. It was concluded that PGBE and IO caused minor embryotoxicity which was not deleterious to growth and survival, and therefore was not teratogenic to offspring of treated rats.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

## TABLE OF CONTENTS

Foreword . . . . . iii
Abstract . . . . .
Tables . . . . .

1. Introduction . . . . . 1

2. Methods. . . . . 1

A. Animals. . . . . 1
B. Dose . . . . . 1
C. Experimental Design. . . . . 2
D. Teratology Study . . . . . 2
E. Postnatal Study. . . . . 4
F. Data Analysis. . . . . 4

3. Results. . . . . 5

A. IO Teratology and Postnatal Studies. . . . . 5
B. PGBE Teratology and Postnatal Studies. . . . . 12
C. Discussion . . . . . 18

References. . . . . 20

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"



EPA-287 (Cin.)
(4-76)

## LIST OF TABLES


| Number | | Page |
|---|---|---|
| 1 | Preparation of Dosing Solutions for 2,4-D Isooctyl (IO) Esters and 2,4-D Propylene Glycol Butyl Ether (PGBE) Esters | 3 |
| 2 | Effect of IO Administered During Organogenesis on Maternal Welfare and Reproduction in Rats. | 6 |
| 3 | Gross Anomalies in Rats Treated During Gestation with IO | 7 |
| 4 | Soft Tissue Anomalies in Rats Treated During Gestation With IO. | 8 |
| 5 | Skeletal Anomalies in Rats Treated During Gestation with IO. | 9 |
| 6 | Effect of IO Administered During Organogenesis on Postnatal Growth and Survival. | 11 |
| 7 | Effect of PGBE Administered During Organogenesis on Material Welfare and Reproduction in Rats. | 13 |
| 8 | Gross Anomalies in Rats Treated During Gestation with PGBE | 14 |
| 9 | Soft Tissue Anomalies in Rats Treated During Gestation with PGBE. | 15 |
| 10 | Skeletal Anomalies in Rats Treated During Gestation with PGBE | 16 |
| 11 | Effect of PGBE Administered During Organogenesis on Postnatal Growth and Survival. | 17 |

## I. INTRODUCTION

The herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). and several of its ester derivatives are commonly used for brush and weed control. The teratogenic potential of these compounds has been investigated in laboratory animals. Fetal pathology and an increased incidence of skeletal anomalies were observed in Wistar rats that received 100 to 150 mg/kg of 2,4-D on days 6 to 15 of gestation.[1] In addition, an increased frequency of skeletal defects was also observed in rats that received butyl, isooctyl, butoxyethanol, and dimethylamine derivatives of 2,4-D.[1]

In another teratology study, doses of 2,4-D up to a maximum tolerated dose of 87.5 mg/kg/day or molar equivalents of 2,4-D isooctyl (IO) esters or 2,4-D propylene glycol butyl ether (PGBE) esters were administered to Sprague-Dawley rats on days 6 to 15 of gestation.[2] The only anomalies which could be related to treatment were decreased fetal body weight, subcutaneous edema, delayed ossification of bone, lumbar ribs, and wavy ribs. Since treatment did not affect fetal or neonatal development and survival, these observations were classified as signs of embryotoxicity and fetotoxicity. Teratogenic effects, which were defined as embryotoxicity which seriously interfered with normal development and survival of the offspring, were not observed.[2]

The present study was undertaken to reevaluate the teratogenic potential of IO and PGBE. The doses of IO and PGBE were selected as the molar equivalents of 0, 6.25, 12.5, 25, and 87.5 mg/kg of 2,4-D.[2] Accordingly, pregnant rats were treated with IO or PGBE on days 6 to 15 of gestation, and their fetuses were examined for defects. In addition, dams from some groups were allowed to deliver, and the growth and development of their pups were monitored.

## II. METHODS

### A. Animals

CD® rats were obtained from the Charles River Breeding Laboratory (Wilmington, Massachusetts) and housed in our animal quarters for at least 7 days prior to use. These quarters are maintained at 22 ± 4°C with a relative humidity of 40 to 60% and a 7 AM to 7 PM photoperiod. The animals were given free access to rodent chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois) and tap water.

### B. Dose

1. Source of test materials: Samples of technical grade 2,4-dichlorophenoxyacetic acid (2,4-D) esters were obtained from the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina. These esters were 2,4-D isooctyl (IO) esters,

(96.6% pure, Dow ARG 172357) and 2,4-D propylene glycol butyl ether (PGBE) esters (97.15% pure, Dow ARG 172358). The samples were received at Midwest Research Institute on May 9, 1980.

2. Calculation of dose: Doses of esters were calculated to be the molar equivalents of 6.25, 12.5, 25, and 87.5 mg/kg of 2,4-D. The molecular weights of 2,4-D, IO, and PGBE are 221, 333, and 358, respectively. Accordingly, for IO the doses of pure ester were 9.44, 18.9, 37.8, and 132 mg/kg, and the doses of technical grade material were 9.77, 19.6, 39.1, and 137 mg/kg. Likewise, for PGBE the doses of pure ester were 10.1, 20.2, 40.5, and 142 mg/kg, and the doses of technical grade material were 10.4, 20.8, 41.7, and 146 mg/kg. Since all doses were given orally in a volume of 2.5 ml/kg, the concentrations of the dosing solutions were calculated accordingly:

$$\text{Concentration} = \frac{\text{mg technical grade}}{\text{kg}} \times \frac{\text{kg}}{2.5 \text{ ml}}$$

For convenience, all doses will be referred to in terms of their equivalent mg/kg level of 2,4-D.

3. Preparation of dose: All doses were prepared by weighing the required amount of corn oil into a bottle, adding the required volume of technical grade ester and mixing. The amount of corn oil and volume of technical grade ester required to prepare 100 ml of the various dosing solutions are presented in Table 1.

## C. Experimental Design

In this study mated rats were divided into nine groups. One group was the control, four groups received IO, and four groups received PGBE. The treated groups received doses of esters which were the molar equivalents of 6.25, 12.5, 25 and 87.5 mg/kg of 2,4-D. All doses were administered from days 6 to 15 of gestation. Dams from each group were sacrificed on day 20 and the fetuses examined for defects. In addition, dams from the control, 12.5 and 87.5 mg/kg, groups were allowed to deliver and the growth and development of their pups were monitored. The study was accomplished in two trials.

## D. Teratology Study

1. Mating: Sexually mature virgin female CD® rats were housed overnight with a proven male breeder. In the morning rats were examined for sperm-positive vaginal smears. The morning when evidence of mating was obtained was identified as day 0 of gestation. Mated animals were weighed, identified by ear tag and randomly assigned to a treatment group.

2. Treatment: All doses were administered orally in a volume of 2.5 ml/kg on days 6 to 15 of gestation. The control group received corn

TABLE 1

PREPARATION OF DOSING SOLUTIONS FOR 2,4-D ISOOCTYL (IO) ESTERS AND 2,4-D PROPYLENE GLYCOL BUTYL ETHER (PGBE) ESTERS

| Ester | Dose (amount/kg)[a] | | Preparation of 100-ml Dosing Solution[a] | |
|---|---|---|---|---|
| | Equivalent[b] | Technical Grade (mg)[c] | Corn Oil (g)[d] | Technical Grade (ml)[e] |
| IO | 6.25 | 9.77 | 91.67 | 0.36 |
| | 12.5 | 19.6 | 91.34 | 0.72 |
| | 25.0 | 39.1 | 90.67 | 1.44 |
| | 87.5 | 137.0 | 87.36 | 5.04 |
| PGBE | 6.25 | 10.4 | 91.66 | 0.37 |
| | 12.5 | 20.8 | 91.32 | 0.74 |
| | 25.0 | 41.7 | 90.64 | 1.48 |
| | 87.5 | 146.0 | 87.22 | 5.19 |

a Required dose in 2.5 ml corn oil.
b Groups that receive the molar equivalent of 6.25, 12.5, 25, and 87.5 mg/kg of 2,4-D.
c Technical grade (mg) = mg ester/purity of technical grade.
Molecular weights (MW) are 2,4-D (221), IO (333), and PGBE (358).
Ester (mg) = mg 2,4-D (MW ester/MW 2,4-D).
Purity of IO is 96.6%; of PGBE, 97.15%.
d Density of corn oil is 0.92 g/ml.
e Density of IO technical grade is 1.085 g/ml.
Density of PGBE technical grade is 1.126 g/ml.

oil and the experimental groups received doses of IO or PGBE which were the molar equivalents of 6.25, 12.5, 25, and 87.5 mg/kg of 2,4-D, as calculated above.

3. Maternal observations: Dams were observed for toxicological signs. In addition, they were weighed on days 0, 6, 13 and 20 of gestation.

4. Fetal observations: Pregnant rats were sacrificed on gestational day 20, a laparotomy was performed, and uterine horns were exposed. The number and position of live, dead, and resorbed fetuses were recorded. Live fetuses were removed, weighed, and immediately examined for external anomalies as described by Wilson.[3]

One-half of the viable fetuses from each litter were dissected and examined for soft tissue anomalies by the free-hand slicing method of Wilson.[3] Each fetus was fixed in 20 to 25 ml of Bouin's fluid for 2 weeks. The hardened fetuses were examined for external anomalies and serially cut from the head through the trunk using a sharp razor blade. No slices were made beyond the kidneys, and the intestines were carefully removed from the pelvic cavity. The cross-sections of the fetuses and the genitourinary organs on the pelvic floor were carefully examined by experienced personnel. The remaining viable fetuses from each litter were processed for skeletal examination. Fetuses were fixed in 70% alcohol for 2 weeks and eviscerated. The fetuses were stored in 1% KOH for 2 days and then stained with alizarin red.[4] After differential decolorization, the skeletons were examined by experienced personnel for anomalies.

## E. Postnatal Study

1. Mating, treatment, and maternal observations: The methodologies for these portions of the study were identical to those described above under Section D, "Teratology Study." Rats for the postnatal study were taken from groups that received 0, 12.5 or 87.5 ME/kg/day PGBE or IO and allowed to deliver.

2. Pup observations: Pups were observed for toxicological signs and on postnatal days 0, 4, 7, 14, and 21.

## F. Data Analysis

Quantitative data are reported as the mean ± standard error. These data were analyzed by Bartlett's test for homogeneity.[5] Homogeneous data were analyzed by Dunnett's procedure or Tukey's omega procedure.[5] Heterogeneous data were analyzed by a nonparametric rank test.[6] The level of statistical significance was selected as $p < 0.05$ unless indicated otherwise. The litter was considered the experimental unit. The percentage of fetuses with a given anomaly was calculated for each litter, and these values were averaged to provide a measure of the affected fetuses per litter.

## III. RESULTS

### A. IO Teratology and Postnatal Studies

#### 1. Teratology study

a. Maternal welfare and reproduction: No mortality was observed in rats treated with up to 87.5 ME/kg/day of IO for 10 days (Table 2). Body weight gain of dams treated with IO was normal throughout gestation. While there was a statistically significant increase in the percent of viable fetuses occurring in the 12.5 ME/kg/day level, the percents of nonviable fetuses were not correspondingly affected in this or any other treated group. In addition, the numbers of fetuses per dam and the fetal body weights were not significantly different from the control group.

b. Gross anomalies: The results of gross observations are summarized in Table 3. External hematomas were present in all groups. Mottled skin was observed in the 6.25, 12.5, and 25.0 ME/kg/day dose levels. Raised cranium was found at the 6.25, 25.0, and 87.5 ME/kg/day levels. In addition, single incidences of reduction anomalies such as reduced lower jaw, shortened digits, and reduced hindquarters were found in fetuses of the 25.0 ME/kg/day dose level. None of the anomalies observed in rats treated with IO occurred at an incidence which was statistically significant.

c. Soft tissue anomalies: Table 4 summarizes the results of the soft tissue examinations of rats treated with IO. While there was no statistically significant increase in any of the observed anomalies, hydronephrosis, both marked and slight, was observed only in the treated animals. The incidence of occluded trachea occurred at a relatively high frequency, but showed no dose-related response and was not significantly increased at any of the treatment levels. In addition, there were single incidences of lateral hydrocephalus and microphthalmia observed in the 6.25 ME/kg/day dose level, and a single incidence of cryptorchid testicle seen at the 87.5 ME/kg/day level. Malformations of the heart, including dextrocardia and/or valvular anomalies, were observed in the 6.25, 12.5 and 25.0 ME/kg/day levels.

d. Skeletal anomalies: The skeletal anomalies observed in fetuses of dams treated with IO are presented in Table 5. For the most part, these anomalies represent minor variations in the degree of ossification of developing fetuses at the time of cesarean section. Of these minor anomalies, there was a significantly increased incidence of lumbar (14th) rib buds in the 87.5 ME/kg/day dose group. In addition, there was a single incidence of major bone malformations including fused ribs, fused centri, and vertical fusion of vertebrae observed in an 87.5 ME/kg/day level litter.

2. Postnatal study: Table 6 presents data on the postnatal growth and survival of pups exposed in utero to IO. There was a significant increase in the postpartum body weights of dams treated with 12.5 mg/kg/day IO from day 0 to day 14 postpartum. This effect, however, reflects an increased body weight of these dams on day 0 of gestation (data not shown).

TABLE 2

EFFECT OF IO ADMINISTERED DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN RATS

| | IO (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number Treated | 37 | 34 | 36 | 28 | 21 |
| Pregnant | 33 | 32 | 34 | 27 | 21 |
| Alive | 33 | 32 | 34 | 27 | 21 |
| Nonpregnant | 4 | 2 | 2 | 1 | - |
| Alive | 4 | 2 | 2 | 1 | - |
| Body Weight (g/rat) | | | | | |
| Day 0 | 246 ± 3[b] | 242 ± 3 | 246 ± 3 | 246 ± 3 | 246 ± 3 |
| 6 | 275 ± 3 | 271 ± 3 | 274 ± 3 | 274 ± 4 | 277 ± 3 |
| 13 | 309 ± 4 | 306 ± 3 | 309 ± 3 | 309 ± 5 | 305 ± 3 |
| 20 | 386 ± 5 | 386 ± 4 | 392 ± 6 | 397 ± 7 | 383 ± 5 |
| Pregnant Survivors | 33 | 32 | 34 | 27 | 21 |
| Implants/Dam | 13.2 ± 0.5 | 13.4 ± 0.4 | 12.7 ± 0.6 | 13.7 ± 0.7 | 12.8 ± 0.6 |
| Viable Fetuses (%) | 94 ± 1 | 95 ± 1 | 98 ± 1[c] | 96 ± 1 | 97 ± 1 |
| Dead Fetuses (%) | 0 | 0 | 0 | 0 | 0 |
| Early Resorptions (%) | 5 ± 1 | 5 ± 1 | 2 ± 1 | 5 ± 1 | 3 ± 1 |
| Late Resorptions (%) | 1 ± 1 | 0 | 0 | 0 | 1 ± 1 |
| Dams with Complete Resorptions | 0 | 0 | 0 | 0 | 0 |
| Live Litters | 33 | 32 | 34 | 27 | 21 |
| Fetuses/Dam | 12.5 ± 0.5 | 12.7 ± 0.5 | 12.5 ± 0.5 | 13.1 ± 0.7 | 12.4 ± 0.6 |
| Males (%) | 52 ± 2 | 47 ± 2 | 57 ± 3 | 58 ± 3 | 50 ± 4 |
| Fetal weight (g) | 3.95 ± 0.05 | 3.94 ± 0.05 | 4.18 ± 0.14 | 3.93 ± 0.09 | 3.83 ± 0.06 |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.
b Mean ± S.E.
c Significantly different from control (Dunnett's test).

TABLE 3

GROSS ANOMALIES IN RATS TREATED DURING GESTATION WITH IO

| | IO (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number of | | | | | |
| Litters Affected/Examined (%) | 14/33 (42) | 14/32 (44) | 12/34 (35) | 16/27 (59) | 11/21 (52) |
| Fetuses Affected/Examined (%) | 24/414 (6) | 16/409 (4) | 26/424 (6) | 28/354 (8) | 24/263 (9) |
| Gross Anomalies | | | | | |
| Immature Skin | 0.3 (1)[b] | 0.2 (1) | 0 (0) | 1.1 (3) | 0.4 (1) |
| Hematoma | 3.9 (11) | 3.1 (11) | 4.4 (11) | 4.4 (14) | 4.9 (8) |
| Mottled Skin | 0 (0) | 0.2 (1) | 1.0 (3) | 0.7 (1) | 0 (0) |
| Light Color to Fetus | 0.2 (1) | 0 (0) | 0.4 (2) | 0.5 (2) | 0 (0) |
| Crescent Shaped Varicose Vein on Side | 0 (0) | 0 (0) | 0.2 (1) | 0 (0) | 0 (0) |
| Raised Cranium | 0 (0) | 0.2 (1) | 0 (0) | 0.6 (1) | 0.3 (1) |
| Exencephalocele | 0 (0) | 0 (0) | 0 (0) | 0.2 (1) | 0 (0) |
| Eye Bulges Absent or Reduced | 0 (0) | 0.2 (1) | 0 (0) | 0 (0) | 0 (0) |
| Reduced Lower Jaw | 0 (0) | 0 (0) | 0 (0) | 0.3 (1) | 0 (0) |
| Protruding Tongue | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.3 (1) |
| Brachydactyly | 0 (0) | 0 (0) | 0 (0) | 0.3 (1) | 0 (0) |
| Hindquarters Reduced | 0 (0) | 0 (0) | 0 (0) | 0.3 (1) | 0 (0) |
| Tail Short | 0.3 (1) | 0 (0) | 0 (0) | 0.3 (1) | 0.4 (1) |
| Tail Kinked | 0.2 (1) | 0 (0) | 0 (0) | 0.3 (1) | 0.4 (1) |
| Runt Small or Dwarf | 1.6 (6) | 0.2 (1) | 0 (0) | 1.6 (5) | 1.8 (4) |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.

b Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parenthesis is the number of affected litters.

TABLE 4

SOFT TISSUE ANOMALIES IN RATS TREATED DURING GESTATION WITH IO

| | IO (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number of | | | | | |
| Litters Affected/Examined (%) | 25/33 (76) | 25/32 (78) | 26/34 (76) | 20/27 (74) | 14/21 (67) |
| Fetuses Affected/Examined (%) | 57/198(28) | 59/196(30) | 63/205(31) | 54/170(32) | 34/125(27) |
| Soft Tissue Anomalies | | | | | |
| Hydrocephalus Lateral | 0 (0)[b] | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) |
| Blood in Ventricles (Brain) | 0.6 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Brain Malformed | 0.6 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Blood in Tissue by Nasal Passage | 0 (0) | 1.5 (2) | 0 (0) | 0 (0) | 0.8 (1) |
| Reduced Eye Bulges | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) |
| Microphthalmia | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) |
| Hemorrhage in Tongue | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) |
| Tongue Protruding from Mouth | 0 (0) | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) |
| Trachea Occluded | 15.2 (15) | 19.8 (17) | 13.2 (15) | 15.3 (12) | 13.0 (10) |
| Displaced Lung | 1.3 (2) | 1.0 (1) | 2.7 (5) | 2.1 (2) | 0.7 (1) |
| Small Lung | 0.5 (1) | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) |
| Dextrocardia | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) |
| Levorotation to Heart | 0 (0) | 0.4 (1) | 0 (0) | 0.8 (1) | 0 (0) |
| Deflated Lung | 0 (0) | 0 (0) | 1.1 (2) | 0 (0) | 0 (0) |
| Agenesis of Left Atrium | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Ventricular Valves Not Fully Formed | 0 (0) | 0.5 (1) | 0.5 (1) | 0 (0) | 0 (0) |
| Aortic Valve with Only Two Cusps | 0 (0) | 0 (0) | 0 (0) | 0.8 (1) | 0 (0) |
| Hemorrhage in Liver | 1.3 (2) | 0.5 (1) | 1.7 (2) | 4.1 (4) | 0.6 (1) |
| Hemorrhage in Abdomen | 0 (0) | 0.5 (1) | 0.7 (1) | 1.3 (2) | 0.7 (1) |
| Liver Hard | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hydronephrosis | 0 (0) | 1.8 (3) | 1.4 (2) | 0.6 (1) | 1.5 (2) |
| Slight Enlargment of Kidney Pelvis | 0 (0) | 1.0 (2) | 1.2 (2) | 0.6 (1) | 1.6 (2) |
| Hypoplastic Kidney | 3.4 (5) | 0.4 (1) | 3.7 (5) | 2.0 (3) | 3.0 (3) |
| Ectopic Kidney | 0 (0) | 0 (0) | 0 (0) | 0.6 (1) | 0 (0) |
| Small Kidney | 0.5 (1) | 0.4 (1) | 0 (0) | 0 (0) | 0.6 (1) |
| Hematoma in Kidney | 0 (0) | 0 (0) | 0.5 (1) | 0.5 (1) | 0 (0) |
| Hydroureter | 1.2 (2) | 2.6 (4) | 2.1 (3) | 0 (0) | 0.7 (1) |
| Distended Urinary Bladder | 2.6 (5) | 1.5 (3) | 3.4 (5) | 1.2 (2) | 2.4 (3) |
| Cryptorchid Testicle | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.8 (1) |
| Malplaced Testicle | 1.0 (2) | 0.5 (1) | 0.4 (1) | 1.1 (2) | 0.8 (1) |
| Malplaced Ovary | 0 (0) | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) |
| Small Fetus | 0.4 (1) | 0.4 (1) | 0 (0) | 1.2 (2) | 0 (0) |
| Subdermal Hemorrhage | 0 (0) | 0.9 (2) | 0 (0) | 0 (0) | 1.6 (2) |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.

b Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parenthesis is the number of affected litters.

TABLE 5

SKELETAL ANOMALIES IN RATS TREATED DURING GESTATION WITH IO

| | IO (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number of | | | | | |
| Litters Affected/Examined (%) | 32/33 (97) | 32/32 (100) | 32/34 (94) | 25/27 (92) | 21/21 (100) |
| Fetuses Affected/Examined (%) | 157/213(74) | 152/212(72) | 130/217(60) | 138/184(75) | 107/134(80) |
| Skeletal Anomalies | | | | | |
| Skull Collapsed Slight | 0.4 (1)[b] | 2.1 (3) | 0.8 (2) | 0 (0) | 0.8 (1) |
| Skull Collapsed Marked | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Basisphenoid Malformed | 0 (0) | 0 (0) | 0 (0) | 0.6 (1) | 0 (0) |
| Basioccipital Incompletely Ossified | 2.6 (3) | 0 (0) | 1.0 (1) | 0 (0) | 0 (0) |
| Squamosal Split | 1.2 (1) | 0.5 (1) | 0 (0) | 0 (0) | 2.5 (3) |
| Jugal Incompletely Ossified | 0 (0) | 0 (0) | 0.9 (1) | 0 (0) | 0 (0) |
| Hyoid Bone Unossified | 5.3 (9) | 6.0 (5) | 2.8 (4) | 2.7 (5) | 4.4 (6) |
| Hyoid Bone Incompletely Ossified | 0.4 (1) | 0 (0) | 0.4 (1) | 0.9 (2) | 1.9 (2) |
| Hyoid Bone Split | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.0 (1) |
| Frontal Bones Incompletely Ossified | 0.4 (1) | 0.9 (2) | 0.4 (1) | 0.5 (1) | 0 (0) |
| Frontal Fontanel Enlarged | 0 (0) | 0 (0) | 0 (0) | 1.1 (2) | 0 (0) |
| Occipital Fontanel Enlarged | 0 (0) | 0 (0) | 0 (0) | 0.6 (1) | 0 (0) |
| Parietals Incompletely Ossified | 2.0 (4) | 0 (0) | 0.8 (2) | 1.0 (2) | 2.1 (2) |
| Interparietal Unossified | 0 (0) | 0 (0) | 0 (0) | 0.6 (1) | 0 (0) |
| Interparietal Incompletely Ossified | 2.2 (3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Interparietals Curved Medially | 0 (0) | 1.5 (3) | 0 (0) | 1.1 (1) | 0 (0) |
| Supraoccipital Unossified | 0 (0) | 0 (0) | 0 (0) | 1.1 (2) | 0 (0) |
| Supraoccipital Incompletely Ossified | 1.2 (1) | 0.5 (1) | 1.2 (2) | 0.5 (1) | 0 (0) |
| Extra Ribs | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) |
| Rib Buds | 1.0 (1) | 3.0 (4) | 5.1 (5) | 0.5 (1) | 9.2 (9)[c] |
| Ribs Unossified | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.7 (1) |
| Incomplete Ossification of Ribs | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.6 (1) |
| Vertically or Malfused Ribs | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.7 (1) |
| Wavy Ribs | 0 (0) | 1.6 (1) | 0 (0) | 0 (0) | 0 (0) |
| Centri Unossified | 0 (0) | 0 (0) | 0 (0) | 1.1 (2) | 0 (0) |
| Centri Lobed | 31.2 (25) | 16.7 (20) | 23.1 (21) | 32.9 (22) | 18.9 (12) |
| Incomplete Ossification of Centri | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Split Centri | 2.1 (5) | 7.2 (11) | 2.5 (4) | 4.5 (7) | 2.0 (3) |
| Vertical Fusion of Centri | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.7 (1) |
| Vertebrae Unossified | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.7 (1) |
| Vertical Fusion of Vertebrae | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0.7 (1) |
| Unossification of Sternebrae | 21.4 (20) | 21.8 (19) | 10.0 (13) | 18.0 (14) | 33.2 (20) |
| Incomplete Ossification of Sternebrae | 24.7 (23) | 29.2 (25) | 20.1 (25) | 28.5 (19) | 36.0 (18) |

TABLE 5 (concluded)

| | IO (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Sternebrae Lobed | 2.4 (2)[b] | 5.8 (7) | 0 (0) | 4.2 (6) | 2.2 (3) |
| Split Sternebrae | 1.3 (2) | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) |
| Sternebrae Fused | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Malalignment of Fusion of Sternebrae | 10.6 (11) | 14.3 (13) | 10.8 (12) | 13.3 (11) | 8.5 (7) |
| Extra Ossification Between Sternebrae | 0 (0) | 0 (0) | 0.3 (1) | 0 (0) | 0 (0) |
| Incomplete Ossification of Ischium | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Pubis Unossified | 0 (0) | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) |
| Pubis Incompletely Ossified | 0.5 (1) | 0.4 (1) | 0 (0) | 0.5 (1) | 0.7 (1) |
| Paws Unossified | 0 (0) | 0 (0) | 0 (0) | 0.6 (1) | 0 (0) |
| Phalanges Unossified | 1.5 (2) | 5.1 (5) | 1.2 (2) | 0.5 (1) | 8.7 (8) |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.
b Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parenthesis is the number of affected litters.
c Significantly different from control (Tukey's procedure).

TABLE 6

EFFECT OF IO ADMINISTERED DURING ORGANOGENESIS ON POSTNATAL GROWTH AND SURVIVAL

| | IO (ME/kg/day)[a] | | |
|---|---|---|---|
| | 0 | 12.5 | 87.5 |
| **No. Viable Litters[b]** | | | |
| 0 | 9[b] | 9 | 9 |
| 4 | 9 | 9 | 9 |
| 7 | 9 | 9 | 8 |
| 14 | 9 | 9 | 8 |
| 21 | 9 | 9 | 8 |
| **No. Pups/Litter** | | | |
| 0 | 13.1 ± 0.4[c] | 12.8 ± 0.8 | 11.2 ± 1.0 |
| 4 | 13.0 ± 0.4 | 12.8 ± 0.8 | 9.7 ± 1.2[d] |
| 7 | 12.8 ± 0.4 | 12.7 ± 0.8 | 9.3 ± 1.5[d] |
| 14 | 12.3 ± 0.4 | 12.3 ± 0.8 | 9.3 ± 1.5 |
| 21 | 12.2 ± 0.3 | 12.3 ± 0.8 | 9.3 ± 1.5 |
| **Dam Weight (g)** | | | |
| 0 | 284 ± 3 | 307 ± 9[d] | 298 ± 3 |
| 4 | 308 ± 6 | 332 ± 7[d] | 312 ± 5 |
| 7 | 321 ± 7 | 350 ± 6[d] | 331 ± 6 |
| 14 | 329 ± 7 | 356 ± 9[d] | 343 ± 6 |
| 21 | 322 ± 10 | 343 ± 10 | 335 ± 9 |
| **Pup Weight (g)** | | | |
| 0 | 6.8 ± 0.1 | 6.7 ± 0.2 | 6.7 ± 0.2 |
| 4 | 10.4 ± 0.3 | 10.4 ± 0.4 | 10.4 ± 0.9 |
| 7 | 15.0 ± 1.0 | 15.0 ± 1.0 | 16.0 ± 3.0 |
| 14 | 26.0 ± 1.0 | 27.0 ± 1.0 | 26.0 ± 4.0 |
| 21 | 40.0 ± 2.0 | 42.0 ± 2.0 | 42.0 ± 7.0 |

a Molar Equivalents (ME) of 0, 12.5 or 87.5 mg/kg of 2,4-D.
b Number of litters with at least one viable pup.
c Mean ± S.E.
d Significantly different from control (Dunnett's test).

Although the number of viable litters was not adversely affected by treatment with IO, the survival of pups from days 0 to 4 was significantly reduced in the 87.5 ME/kg/day dose level; this was due primarily to the loss of one complete litter. In contrast, the growth of pups in both groups treated with IO was normal.

B. PGBE Teratology and Postnatal Studies

1. Teratology study

a. Maternal welfare and reproduction: No mortality was observed in rats treated with PGBE (Table 7). Dams treated with 25.0 ME/kg/day PGBE had significantly reduced body weights during gestation. However, since dams in this group had reduced body weights on day 0 of gestation, this effect was determined not to be treatment-related. Moreover, neither the number of implants per dam nor the number of fetuses per dam were affected by treatment with PGBE. In addition, there was no evidence of fetal toxicity as monitored by percent viable fetuses and fetal body weight.

b. Gross anomalies: There were few gross anomalies observed in the PGBE group (Table 8). External hematomas were most frequently observed and occurred at all dose levels including the control group.

c. Soft tissue anomalies: The soft tissue anomalies observed in fetuses of dams treated with PGBE are presented in Table 9. Hydronephrosis, both marked and slight, as well as subdermal hemorrhage, though not dose-related nor statistically significant, was observed in only the treated groups. In addition, there was a high frequency of the incidence of occluded trachea. This anomaly as well showed no dose-related response nor was it statistically significant. Other soft tissue anomalies occurring were in low frequency and seemed to appear by random variation.

d. Skeletal anomalies: The skeletal anomalies are shown in Table 10. The skeletal anomalies observed represent minor variations in the degree of ossification at the time of caesarian section. However, there was a significantly increased incidence of lumbar (14th) rib buds observed in the 87.5 ME/kg/day treatment group. None of the other anomalies occurred at an incidence which reached a level of statistical significance, and no major morphological malformations were observed.

2. Postnatal study: Pups from dams treated with PGBE during organogenesis grew at a normal rate during lactation (Table 11). Also, there were no significant differences in the numbers of pups per litter. Maternal body weight in the treated groups, while significantly increased at parturition, were similar to the controls throughout the remainder of the lactation period.

TABLE 7

EFFECT OF PGBE ADMINISTERED DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN RATS

| | PGBE (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number Treated | 37 | 37 | 36 | 28 | 19 |
| Pregnant | 33 | 36 | 34 | 28 | 15 |
| Alive | 33 | 36 | 34 | 28 | 15 |
| Nonpregnant | 4 | 1 | 2 | 0 | 4 |
| Alive | 4 | 1 | 2 | 0 | 4 |
| Body Weight (g/rat) | | | | | |
| Day 0 | 246 ± 3[b] | 240 ± 3 | 239 ± 3 | 234 ± 3[c] | 239 ± 5 |
| 6 | 275 ± 3 | 272 ± 4 | 268 ± 4 | 264 ± 4 | 269 ± 5 |
| 13 | 309 ± 4 | 304 ± 4 | 299 ± 3 | 295 ± 4[c] | 304 ± 5 |
| 20 | 386 ± 5 | 380 ± 5 | 378 ± 5 | 365 ± 6[c] | 381 ± 8 |
| Pregnant Survivors | 33 | 36 | 34 | 28 | 15 |
| Implants/Dam | 13.2 ± 0.5 | 12.4 ± 0.6 | 12.6 ± 0.5 | 11.7 ± 0.8 | 12.7 ± 0.8 |
| Viable Fetuses (%) | 94 ± 1.5 | 96 ± 1.0 | 96 ± 1.0 | 91 ± 3.6 | 95 ± 1.2 |
| Dead Fetuses (%) | 0 | 0 | 0 | 0 | 0 |
| Early Resorptions (%) | 5 ± 1 | 4 ± 1 | 4 ± 1 | 7 ± 3 | 5 ± 1 |
| Late Resorptions (%) | 1 ± 1 | 0 | 0 | 0 | 0 |
| Dams with Complete Resorptions | 0 | 0 | 0 | 0 | 0 |
| Live Litters | 33 | 36 | 34 | 28 | 15 |
| Fetuses/Dam | 12.5 ± 0.5 | 11.9 ± 0.6 | 12.0 ± 0.4 | 10.4 ± 0.8 | 12.1 ± 0.7 |
| Males (%) | 52 ± 2 | 54 ± 3 | 53 ± 2 | 44 ± 4 | 44 ± 4 |
| Fetal Weight (g) | 3.95 ± 0.05 | 4.01 ± 0.06 | 4.02 ± 0.06 | 3.95 ± 0.06 | 3.84 ± 0.09 |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.
b Mean ± S.E.
c Significantly different from control (Dunnett's test).

TABLE 8

GROSS ANOMALIES IN RATS TREATED DURING GESTATION WITH PGBE

| | PGBE (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number of | | | | | |
| Litters Affected/Examined (%) | 14/33 (42) | 15/36 (42) | 16/34 (47) | 9/28 (32) | 7/15 (47) |
| Fetuses Affected/Examined (%) | 24/414 (6) | 34/429 (8) | 26/409 (6) | 12/292 (4) | 13/181 (7) |
| Gross Anomalies | | | | | |
| Immature Skin | 0.3 (1)[b] | 0 (0) | 0 (0) | 1.5 (2) | 0 (0) |
| Hematoma | 3.9 (11) | 3.9 (11) | 5.3 (13) | 3.6 (9) | 5.6 (7) |
| Mottled Skin | 0 (0) | 0.7 (3) | 0 (0) | 0 (0) | 0 (0) |
| Light Color | 0.2 (1) | 0.2 (1) | 0.5 (2) | 0.2 (1) | 0.9 (2) |
| Crescent Shaped Varicose Vein on Side | 0 (0) | 0 (0) | 0.2 (1) | 0 (0) | 0 (0) |
| Reduced Snout | 0 (0) | 0.2 (1) | 0 (0) | 0 (0) | 0 (0) |
| Reduced Lower Jaw | 0 (0) | 0 (0) | 0 (0) | 1.2 (1) | 0 (0) |
| Protruding Tongue | 0 (0) | 1.4 (1) | 0 (0) | 0 (0) | 0 (0) |
| Tail Short | 0.3 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Tail Kinked | 0.2 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Runt | 1.6 (6) | 0.9 (3) | 0.5 (2) | 2.0 (4) | 0 (0) |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.
b Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parenthesis is the number of affected litters.

TABLE 9

SOFT TISSUE ANOMALIES IN RATS TREATED DURING GESTATION WITH PGBE

| | PGBE (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number of | | | | | |
| Litters Affected/Examined (%) | 25/33 (76) | 24/36 (67) | 26/34 (76) | 22/28 (78) | 10/15 (67) |
| Fetuses Affected/Examined (%) | 57/198(29) | 46/206(22) | 59/198(30) | 47/138(34) | 16/86 (19) |
| Soft Tissue Anomalies | | | | | |
| Hydrocephalus Lateral | 0 (0)[b] | 0.3 (1) | 0 (0) | 0 (0) | 0 (0) |
| Superior Sagittal Sinus Enlarged | 0 (0) | 0 (0) | 1.2 (1) | 0 (0) | 0 (0) |
| Blood in Ventricles (Brain) | 0.6 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Brain Malformed | 0.6 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Blood in Eye | 0 (0) | 0.6 (1) | 0 (0) | 0 (0) | 0 (0) |
| Lens Absent | 0 (0) | 0 (0) | 0 (0) | 1.9 (1) | 0 (0) |
| Tongue Protruding from Mouth | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) |
| Cleft Palate | 0 (0) | 0 (0) | 0 (0) | 1.9 (1) | 0 (0) |
| Trachea Occluded | 15.2 (15) | 16.1 (18) | 16.4 (19) | 22.6 (12) | 10.4 (5) |
| Agenesis of Lung | 0 (0) | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) |
| Displaced Lung | 1.3 (2) | 0 (0) | 0.4 (1) | 2.0 (2) | 0 (0) |
| Small Lung | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Deflated Lung | 0 (0) | 0 (0) | 0 (0) | 1.4 (2) | 0 (0) |
| Innominate Artery Short | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) |
| Agenesis of Left Atrium | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hemorrhage in Liver | 1.3 (2) | 0.4 (1) | 0.4 (1) | 0.6 (1) | 1.1 (1) |
| Hemorrhage in Abdomen | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) | 1.1 (1) |
| Liver Hard | 0.5 (1) | 0 (0) | 0 (0) | 0.7 (1) | 1.1 (1) |
| Blood in Stomach | 0 (0) | 0 (0) | 0.6 (1) | 0 (0) | 0 (0) |
| Hydronephrosis | 0 (0) | 1.4 (2) | 3.2 (5) | 1.9 (3) | 1.7 (1) |
| Slight Enlargement of Kidney Pelvis | 0 (0) | 0.9 (2) | 0 (0) | 0.5 (1) | 1.0 (1) |
| Kidney Pelvis Assymetrical | 3.4 (5) | 0.4 (1) | 0.8 (2) | 2.6 (3) | 0 (0) |
| Small Kidney | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Hematoma in Kidney | 0 (0) | 0 (0) | 1.0 (1) | 0 (0) | 0 (0) |
| Hydroureter | 1.2 (2) | 0.9 (1) | 0 (0) | 0 (0) | 0 (0) |
| Slight Enlargement of Ureter | 0 (0) | 0.9 (1) | 0 (0) | 0 (0) | 0 (0) |
| Distended Urinary Bladder | 2.6 (5) | 0.7 (2) | 3.4 (4) | 1.4 (2) | 1.9 (2) |
| Monorchid | 0 (0) | 0.3 (1) | 0 (0) | 0 (0) | 0 (0) |
| Malplaced Testicle | 1.0 (2) | 1.1 (3) | 1.3 (3) | 1.2 (2) | 0 (0) |
| Misshapen Uterus | 0 (0) | 0 (0) | 0 (0) | 0.5 (1) | 0 (0) |
| Small Fetus | 0.4 (1) | 1.5 (2) | 0 (0) | 3.6 (4) | 0 (0) |
| Reduced Lower Jaw | 0 (0) | 0 (0) | 0 (0) | 1.9 (1) | 0 (0) |
| Subdermal Hemorrhage | 0 (0) | 0.3 (1) | 1.9 (3) | 0.5 (1) | 1.0 (1) |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.

b Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parenthesis is the number of affected litters.

TABLE 10

SKELETAL ANOMALIES IN RATS TREATED DURING GESTATION WITH PGBE

| | PGBE (ME/kg/day)[a] | | | | |
|---|---|---|---|---|---|
| | 0 | 6.25 | 12.5 | 25 | 87.5 |
| Number of | | | | | |
| Litters Affected/Examined (%) | 32/33 (97) | 34/36 (94) | 32/34 (94) | 27/28 (96) | 15/15(100) |
| Fetuses Affected/Examined (%) | 157/213(74) | 157/223(70) | 140/211(66) | 107/153(70) | 71/95 (75) |
| Skeletal Anomalies | | | | | |
| Skull Collapsed Slight | 0.4 (1)[b] | 0.3 (1) | 0 (0) | 0.6 (1) | 1.0 (1) |
| Skull Collapsed Marked | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Basioccipital Incompletely Ossified | 2.6 (3) | 2.5 (2) | 0 (0) | 1.0 (1) | 0 (0) |
| Squamosal Split | 1.2 (1) | 0 (0) | 0 (0) | 0 (0) | 1.9 (1) |
| Squamosal Incompletely Ossified | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) |
| Hyoid Bone Unossified | 5.3 (9) | 2.4 (4) | 5.6 (8) | 4.9 (7) | 7.5 (4) |
| Hyoid Bone Incompletely Ossified | 0.4 (1) | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) |
| Frontal Bones Incompletely Ossified | 0.4 (1) | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) |
| Parietals Incompletely Ossified | 2.0 (4) | 0.5 (1) | 1.0 (2) | 0 (0) | 3.8 (1) |
| Interparietal Incompletely Ossified | 2.2 (3) | 0 (0) | 0 (0) | 0.7 (1) | 0 (0) |
| Interparietals Curved Medially | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.0 (1) |
| Supraoccipital Incompletely Ossified | 1.2 (1) | 0 (0) | 0 (0) | 0 (0) | 1.9 (1) |
| Extra Ribs | 0 (0) | 0 (0) | 0 (0) | 0.9 (1) | 0 (0) |
| Rib Buds | 1.0 (1) | 6.3 (7) | 2.2 (3) | 2.1 (3) | 13.3 (8)[c] |
| Ribs Unossified | 0 (0) | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) |
| Wavy Ribs | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.9 (1) |
| Centri Lobed | 31.2 (25) | 22.2 (23) | 22.0 (18) | 33.4 (18) | 19.6 (8) |
| Incomplete Ossification of Centri | 0.4 (1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Split Centri | 2.1 (5) | 1.2 (3) | 3.6 (6) | 4.7 (5) | 3.0 (3) |
| Incomplete Ossification of Vertebrae | 0 (0) | 0.3 (1) | 0 (0) | 0 (0) | 0 (0) |
| Unossification of Sternebrae | 21.4 (20) | 19.0 (17) | 14.6 (18) | 22.3 (12) | 14.1 (8) |
| Incomplete Ossification of Sternebrae | 24.7 (23) | 27.0 (25) | 26.5 (28) | 23.5 (17) | 36.6 (11) |
| Sternebrae Lobed | 2.4 (2) | 8.0 (7) | 1.9 (4) | 5.3 (4) | 3.3 (1) |
| Split Sternebrae | 1.3 (2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Sternebrae Fused | 0.4 (1) | 0 (0) | 0 (0) | 0.4 (1) | 0 (0) |
| Malalignment of Fusion of Sternebrae | 10.6 (11) | 10.3 (15) | 10.1 (11) | 12.5 (9) | 8.9 (4) |
| Ischium Incompletely Ossified | 0.5 (1) | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) |
| Pubis Unossified | 0 (0) | 0.5 (1) | 0 (0) | 0 (0) | 0 (0) |
| Pubis Incompletely Ossified | 0.5 (1) | 0.5 (1) | 0 (0) | 0.6 (1) | 1.0 (1) |
| Phalanges of Paws Unossified | 1.5 (2) | 0.7 (1) | 5.1 (6) | 3.0 (1) | 1.8 (2) |

a Molar Equivalents (ME) of 0, 6.25, 12.5, 25, or 87.5 mg/kg of 2,4-D.

b Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis. The number in parenthesis is the number of affected litters.

c Significantly different from control (Tukey's procedure).

TABLE 11

EFFECT OF PGBE ADMINISTERED DURING ORGANOGENESIS ON POSTNATAL GROWTH AND SURVIVAL

| | PGBE (ME/kg/day)[a] | | |
|---|---|---|---|
| | 0 | 12.5 | 87.5 |
| **No. Viable Litters[b]** | | | |
| Day 0 | 9 | 9 | 9 |
| 4 | 9 | 9 | 9 |
| 7 | 9 | 9 | 9 |
| 14 | 9 | 9 | 9 |
| 21 | 9 | 9 | 9 |
| **No. Pups/Litter** | | | |
| Day 0 | 13.1 ± 0.4[c] | 12.9 ± 0.6 | 11.6 ± 0.7 |
| 4 | 13.0 ± 0.4 | 12.9 ± 0.6 | 11.7 ± 0.7 |
| 7 | 12.8 ± 0.4 | 12.8 ± 0.7 | 11.6 ± 0.7 |
| 14 | 12.3 ± 0.4 | 12.7 ± 0.6 | 11.2 ± 0.6 |
| 21 | 12.2 ± 0.3 | 12.6 ± 0.6 | 11.2 ± 0.6 |
| **Dam Weight (g)** | | | |
| Day 0 | 284 ± 3 | 306 ± 5[d] | 302 ± 6[d] |
| 4 | 308 ± 6 | 318 ± 6 | 322 ± 6 |
| 7 | 321 ± 7 | 337 ± 6 | 336 ± 6 |
| 14 | 329 ± 7 | 343 ± 3 | 339 ± 6 |
| 21 | 322 ± 10 | 334 ± 5 | 339 ± 11 |
| **Pup Weight (g)** | | | |
| Day 0 | 6.8 ± 0.1 | 6.5 ± 0.2 | 6.5 ± 0.2 |
| 4 | 10.4 ± 0.3 | 10.4 ± 0.4 | 10.9 ± 0.5 |
| 7 | 14.7 ± 0.6 | 14.4 ± 0.6 | 15.0 ± 0.7 |
| 14 | 25.7 ± 1.1 | 25.6 ± 1.1 | 27.3 ± 1.7 |
| 21 | 40.0 ± 2.0 | 39.0 ± 2.0 | 42.0 ± 3.0 |

a Molar Equivalents (ME) of 0, 12.5 or 87.5 mg/kg of 2,4-D.
b Number of litters with at least one viable pup.
c Mean ± S.E.
d Significantly different from control (Dunnett's test).

C. Discussion

Pregnant rats were exposed to 0, 6.25, 12.5, 25.0 or 87.5 ME/kg/day of IO or PGBE for 10 days starting on day 6 of gestation. Mortality was not observed in rats receiving up to 87.5 ME/kg/day IO or PGBE. While rats which received 25.0 ME/kg/day had significantly reduced body weights during gestation, this was determined not to be treatment-related since this effect occurred before treatment began. Further, all other dams gained weight normally during gestation.

Embryo or fetal lethality, as measured by percent viable fetuses, percent early resorptions, number of dams with complete resorptions, and fetal weight, was not observed with IO or PGBE.

None of the gross anomalies observed in rats were increased to a statistically significant level by treatment with either PGBE or IO.

Soft tissue anomalies observed in rats treated with either PGBE or IO were not increased to a significant level. However, hydronephrosis, both marked and slight, was observed only in the treated animals. This anomaly, though, occurred at very low incidences in all dose levels in rats treated with both PGBE and IO, and showed no dose-related response.

Skeletal anomalies observed in rats treated with PGBE or IO consisted mainly of minor variations in the degree of ossification. There was a significantly increased number of fetuses with lumbar (14th) rib buds in the 87.5 ME/kg/day dose levels of both IO and PGBE. Additionally, in one pup treated with 87.5 ME/kg/day of IO some major bone malformations of the axial skeleton, including fused ribs, fused centri, unossified vertebrae, and fusion of vertebrae, were observed.

These scattered effects are considered to be within normal biological variation. When the significance level is set at $p < 0.05$, it is expected that one anomaly in twenty in each dose group will be significantly different by random variation. The differences observed here were not dose-related and did not include clusters of related anomalies. Therefore, these differences have been considered toxicologically unimportant.

In addition, a postnatal growth and survival study was performed on rats receiving 0, 12.5, or 87.5 ME/kg/day PGBE or IO. Pup body weights for both compounds were normal. In rats receiving 87.5 ME/kg/day IO, there was a reduced number of pups per litter on days 4 and 7 postpartum. This effect, however, represents a whole litter loss by one of the dams at this dose level. Since the survival of pups from other dams in this treatment group was similar to the control, it was concluded that this effect was not treatment-related.

In a previous study,[2] PGBE and IO were given orally to rats in similar doses on days 6 through 15 of gestation, and an increased incidence of fetuses with subcutaneous edema was observed. This anomaly was not observed in the present study in any of the dose levels studied. However, the same study[2] reported a significant increase in the incidence of lumbar

ribs in the 87.5 ME/kg/day dose levels of both IO and PGBE. An increase in the appearance of lumbar ribs was also reported in another study[1] using Wistar rats at only slightly higher maximum doses of PGBE and IO.

Although the occurrence of lumbar ribs has no morphological or functional consequence to the fetuses and appears spontaneously in control animals, it falls within the scope of embryotoxicity.[7] Since this effect is not detrimental to the normal development and survival of fetuses, it is not classified as a "teratogenic" effect.[7]

In summary, the results of this study indicate that (a) PGBE or IO produce no adverse effects on maternal welfare or pup viability; (b) PGBE or IO exhibit embryotoxicity of insignificant detrimental effect; and (c) PGBE or IO do not adversely affect postnatal growth and survival of pups.

Accordingly, these results indicate that PGBE and IO are not teratogenic in CD® rats.

## REFERENCES


1. Khera, K. S., and W. P. McKinley, "Pre- and Postnatal Studies on 2,4,5-Trichlorophenoxyacetic Acid, 2,4-Dichlorophenoxyacetic Acid and Their Derivatives in Rats," Toxicol. Appl. Pharmacol., 22:14-28 (1972).

2. Schwetz, B. A., G. L. Sparschu, and P. J. Gehring, "The Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Esters of 2,4-D on Rat Embryonal, Foetal and Neonatal Growth and Development," Fd. Cosmet. Toxicol., 9: 801-817 (1971).

3. Wilson, J. G., "Methods for Administering Agents and Detecting Malformations in Experimental Animals," in Teratology--Principles and Techniques, J. G. Wilson and J. Warkany (eds.), University of Chicago Press, Chicago, Illinois, pp. 262-277 (1965).

4. Staples, R. C., and V. L. Schnell, "Refinements in Rapid Clearing Techniques in the KOH-Alizarin Red S Method for Fetal Bones," Stain Technol., 39:61-63 (1964).

5. Steel, R. G. D., and J. H. Torrie, Principles and Procedures of Statistics, McGraw-Hill Book Company, New York (1960).

6. Mann, H. B., and D. R. Whitney, "On a Test of Whether One of Two Random Variables is Stochastically Larger than the Other," Ann. Math. Stat., 18:50-60 (1947).

7. Wilson, J. G., Environment and Birth Effects, Academic Press, New York (1973).